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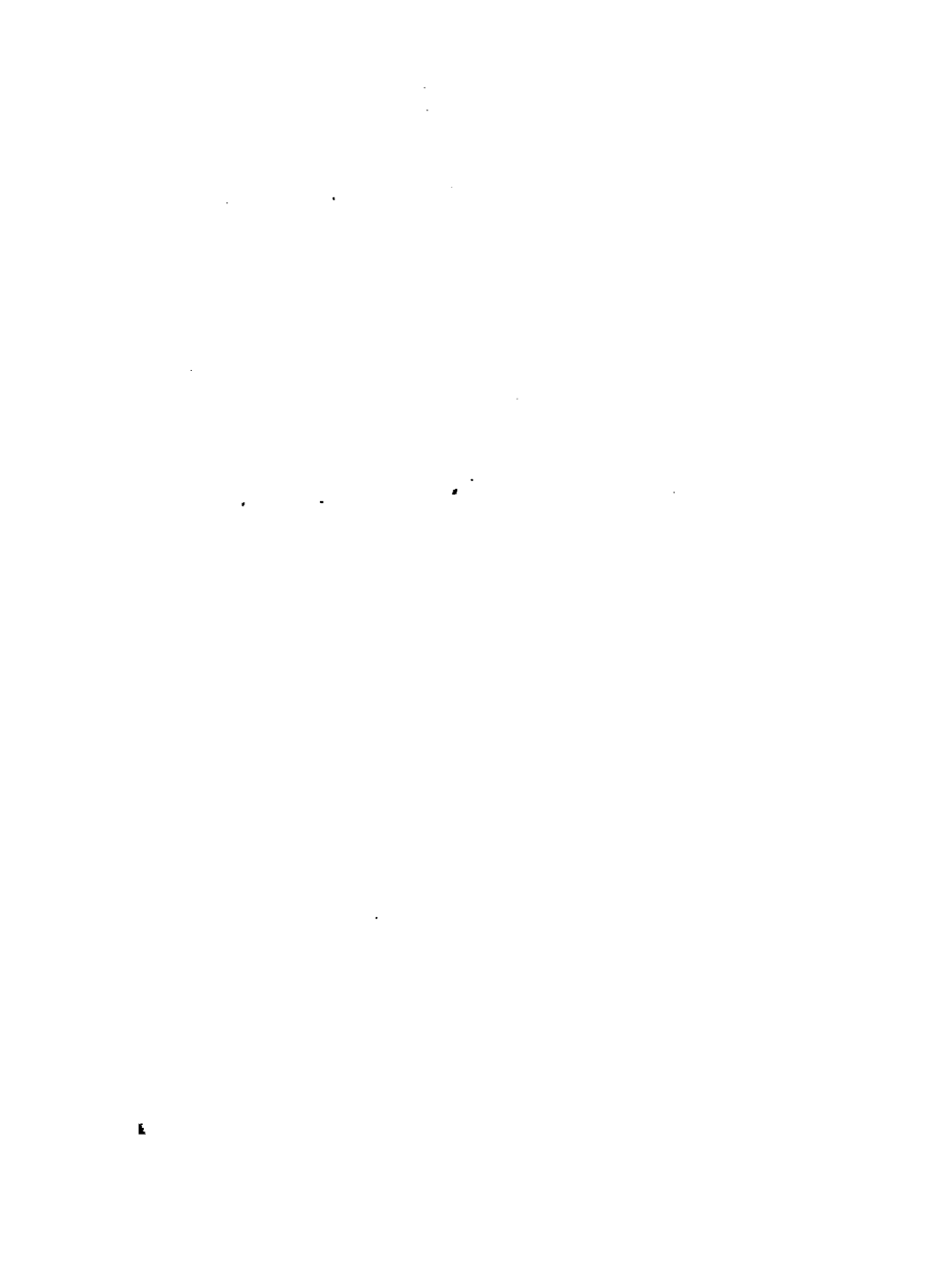
SECTION-CUTTING

— — —  
D<sup>R</sup> MARSH

sufficiently firm to be cut with ease. Sections may be made with a razor kept constantly wetted with water, or, if the preservation of colour be no object, methylated spirit may be employed for the purpose. As the subsequent treatment of such sections in no wise differs from that required by those cut in the microtome, we shall defer its consideration until that method of section has been described (§ 12).

3. *On Cutting unprepared Animal Tissues.*—For the cutting of fresh *animal* tissues several plans may be followed. Thus, if a section of only very limited area be required, it may be obtained by snipping a piece off the tissue with a pair of bent scissors, which, for this purpose, are so made that the blades are *curved on the flat* (Carpenter). If this be carefully performed it will be found that a large portion of the section (particularly at the circumference) so obtained will be sufficiently thin for examination. If a larger section be desired, an attempt may be made to cut it with a very sharp scalpel or razor, the blade of which whilst in use must be kept *flooded* with water or spirit, the latter of which is to be preferred. Recourse may also be had to *Valentin's* knife. This consists of two long, narrow blades, running parallel to each other, the distance at which the blades are held apart, and which, of course,





## **SECTION-CUTTING**



# SECTION-CUTTING

A PRACTICAL GUIDE

TO THE

PREPARATION AND MOUNTING OF SECTIONS  
FOR THE MICROSCOPE

*SPECIAL PROMINENCE BEING GIVEN TO THE  
SUBJECT OF ANIMAL SECTIONS*

BY

DR SYLVESTER MARSH

*WITH ILLUSTRATIONS*

LONDON

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—  
1878

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## P R E F A C E.

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IF we glance at any of the numerous magazines devoted either wholly or in part to the subject of microscopy, we shall hardly fail to be struck with the numerous queries relating to SECTION-CUTTING, which are to be found in its pages. A simple explanation of this widespread want is afforded by the fact that the use of the microscope has at the present day extended to (and is still rapidly spreading amongst) vast numbers of students, who, in many instances, possess neither the leisure nor the means to refer for information to large and expensive text-books. Moreover, were they actually to consult such works, they would practically fail to obtain the information of which they are in need, for the coveted instruction is to be found in those treatises only in a scattered and fragmentary form—no

work with which we are acquainted treating of the subject in anything like a detailed manner. To fill this *vacuum* in the literature of microscopy the present manualette has been prepared. Little claim is made to originality, yet the book is by no means a mere compilation, but the outcome of long and extensive personal experience in the cutting and mounting of microscopical sections. Every process described has been put to the test of actual trial, so that its worth may confidently be depended upon. Many of the little points insisted upon in the ensuing pages will doubtless to the practised microscopist appear superfluous or even puerile; but a vivid recollection of our own early failures and disappointments assures us that it is just these very *minutiae* of detail which will be found most serviceable in directing and sustaining the faltering footsteps of the tyro.

St. HELENS,

*September, 1878.*

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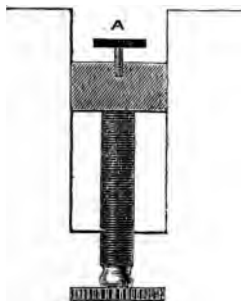
## SECTION-CUTTING.



### PART I.

1. *Introduction.*—Many of the most interesting objects with which the microscopist has to deal, cannot be made to reveal their beauty or minute structure until they have been cut into slices or *sections*, of such a degree of thinness as to render them transparent, and thus permit of their examination by transmitted light, with objectives of varying power. Unfortunately, however, very few of the objects of this class are, in their natural condition, in a suitable state to be submitted to this method of procedure. Some are of such a soft and yielding nature that any attempt to cut them is an utter failure, for in place of a perfect section being obtained, nothing remains upon the knife but a mass of diffuent pulp; others, again, are of such density as to resist the action of any cutting instrument.

many instruments, of otherwise great merit, have their efficiency seriously impaired. Fortunately, this imperfection is easily remedied, all that is required being that the upper surface of the plug should be furnished with some kind of projection, having at its summit a table-like expansion, as shown at A in the Figure. The imbedding paraffine, by penetrating beneath and around this, becomes firmly attached to the plug, and thus all risk of its rising is effectually avoided. If the student wishes to secure a really first-class instrument, none can be so



SECTION OF MICROTOME-TUBE SHOWING ARRANGEMENT (A)  
TO PREVENT "RISE" OF PARAFFINE.

confidently recommended as the freezing microtome of Professor Rutherford. In addition to its being the best instrument for carrying out the freezing method (§ 18), this machine is equally effective for cutting tissues imbedded

in paraffine, or any of the other agents used for that purpose; indeed, whatever work a microtome can do, *this one* will perform.

9. *Æther Microtome*.—A word here as to freezing microtomes, where the agent used is æther. Such as have fallen under our notice have not answered the expectations we were justly entitled to form of them. That it is possible to freeze a piece of tissue by their use is undeniable, but it is, as a rule, at an expenditure of such a quantity of æther (only the very best of which must be used) as to constitute it a very expensive proceeding. Another serious disadvantage they possess is, that if the supply of æther be intermitted for only a very short time, the already frozen tissue thaws with great and most inconvenient rapidity.

10. *Section-Knife*.—Of not less importance than the microtome is the section-knife, to be used in conjunction with it. How perfect soever the former, and whatever the dexterity of the operator, unless he be provided with a suitable and well-made knife, he will never succeed in obtaining satisfactory results. As to the most desirable *size* of the knife, much difference of opinion seems to exist, section knives varying in this respect from a blade of extreme shortness to one which fell under our observation, in which the portentous length of



*thirteen* inches was attained. What advantages were to be expected by prolonging the blade to this extravagant length, must remain an inscrutable mystery to all save its designer. Concerning the *shape* of the knife, it is frequently advised that the surface which has to glide along the cutting-plate of the microtome should be ground *flat*. A most unsuitable arrangement, as a very little actual experience of section-cutting will speedily demonstrate. After many unsuccessful attempts to obtain a really good and reliable section-knife, we determined to have one specially made, which, as it has proved everything that could be desired, merits a brief description. It is of the utmost importance that the blade be made of good and well-tempered steel, not only that it may be capable of receiving an edge of the most exquisite keenness, but also that it may *retain it*. The knife of which we speak (and which was made by Mr. Gardner, of South Bridge, Edinburgh) is furnished with a blade *four inches* long, and  $\frac{7}{8}$ -inch broad, set into a square handle of boxwood, also four inches in length. The thickness of the blade at the back is not quite  $\frac{1}{4}$ -inch, whilst *both* of its surfaces are slightly hollow ground. It is essentially necessary that the back and edge of the blade be *strictly* parallel to each other, otherwise the

knife, when in use, will have such a tendency to tilt over as to render its management extremely difficult. It is very easy to discover if this condition be fulfilled, for if on carefully laying the flat of the blade upon a piece of level glass, every portion of both back and edge are found to be in close contact with it, the knife may in this respect be considered perfect. Every student who aspires to be a successful section-cutter should provide himself with a good Turkey oilstone, *and learn to use it*. He should also possess a razor strop, as it will be in constant requisition. It may here be remarked that though *razors*, as a rule, are unsuitable for use with the microtome from want of uniformity in the thickness of their blades, yet, if only a small object is to be cut—for instance, a thin root or stem—very good results may be obtained from their use, especially if one of the old-fashioned make, having a thick back and slightly *concave* surfaces, be employed.

11. *Imbedding in Paraffine for Microtome.*—Having described at some length the various instruments necessary for section-cutting, we will now consider how they are to be used. Let us endeavour to cut some sections—say of a piece of kidney—and in so doing we will adopt the “paraffine” method of imbedding.

Ordinary paraffine, however, when used alone, is rather too hard for our purpose. In order, therefore, to bring it to a suitable consistence, it must be mixed with one-fifth its weight of common unsalted lard, a gentle heat applied, and the two thoroughly stirred together. A quantity of this should be prepared, so that it may always be ready when wanted—it is very conveniently kept in an ointment pot or preserve jar, the top of the latter being well covered, to keep out the dust. When it is intended to use this mixture for the purpose of imbedding, only just about the quantity required should be melted ; for in doing this it is advisable to use as low a degree of heat as possible, not only to prevent injury to the tissue to be imbedded, but also that the paraffine when cooling may not undergo such an amount of contraction as to cause it to shrink from the sides of the microtome tube. It is therefore a good plan to effect the melting in a water-bath, a simple kind of which, something after the fashion of a glue-pot, would be made for a few pence by any tinman.

The kidney which we are about to cut has, of course, gone through the process of hardening already described (§ 5), and is now preserved in spirit. A small piece, say half an inch square, is selected, removed with forceps, *and placed* on a bit of blotting paper, when the

surface of the tissue will rapidly become dry (*only the surface* must be allowed to dry). It is the usual plan now to proceed at once to imbed it in the melted paraffine. This is a most undesirable step, and gives rise at a later stage of our proceedings to a great amount of trouble and annoyance, for after sections have been cut from a tissue so imbedded it will be found that portions of paraffine adhere to their edges with such tenacity that in the case of many of them there is no effectual method of removing the paraffine, short of soaking the sections in warm æther; a very objectionable proceeding, for though the æther will undoubtedly remove the paraffine, it will also dissolve out any fatty matters which the section itself may naturally contain. All this annoyance may be prevented by subjecting the tissue to a simple preparatory treatment before it is imbedded in the paraffine. For this purpose prepare a very *weak* solution of gum arabic in water—twenty grains to the ounce. Into this, by means of the forceps, dip for a few moments the already *surface-dried* tissue, taking special care not to squeeze it, or the pressure will cause the spirit from its interior to remoisten the surface, which would prevent the gum from adhering. We shall see the value of this a little later on. Remove the tissue from the solution on to blotting paper,

when the superfluous gum will speedily drain off, and in two or three minutes the *surface* will have become quite glazy and dry. Having melted some paraffine mixture in the water-bath, the tissue held in the forceps must be plunged for an instant into the heated liquid and immediately withdrawn, when the crust of paraffine with which it is enveloped will promptly harden. Whilst this is taking place we may make ready the microtome. Having by means of the milled head or handle depressed the plug in the tube so as to leave a free opening about an inch deep at its upper end, we must pour in the melted paraffine, which by this time will have become a little cooler, until the cavity be about half filled. The prepared tissue must now be introduced, care being taken to place it in such a position that the sections may be cut in the desired direction. The tissue must, if necessary, be held in position with forceps or a needle point, till the imbedding material becomes hard enough to give it due support. It is here to be remembered that it will not be advisable to place the tissue in the centre of the tube—it will be much more easily cut if placed rather nearer to that edge of the tube which is situated next the operator in the act of cutting. *More paraffine* is to be slowly added, until the

tissue is completely covered ; even after this still more should be added, for it will be found that in cooling the paraffine shrinks so as to leave a cup-shaped depression in its centre, whereby portions of the tissue which were previously covered are again laid bare. The best method of preventing this is to use the paraffine at as low a temperature as possible, and to use plenty of it. The microtome, with its contents, must now be removed to a cool place, when the paraffine will soon become solidified. Whilst this is being accomplished we may make our further preparations. The first thing we require will be a large basin, full of freshly filtered water, and provided with a cover. A small beaker of methylated spirit, with a dipping rod or pipette, will also be necessary. We must now see that the section-knife is in thorough order, to ensure which it will be advisable to give it a few turns on the stop. An ordinary razor will also be of service.

12. *Employment of Microtome.*—The paraffine being sufficiently hard, we will clamp the microtome on to the table, and seat ourselves on a chair of convenient height before it. To our right stand the basin of water, razor, and section-knife ;—the beaker of spirit to the left, and a cloth on our knee. A few turns of the microtome screw having brought the

paraffine to the surface, a thick slice is to be cut off, and this repeated until the imbedded tissue comes into view. This preliminary work had best be done with the razor, as it is needless to subject our section-knife to unnecessary wear and tear. By a fractional revolution of the screw the tissue is now slightly elevated, and with the pipette held in the left hand, a large drop of spirit is to be let fall upon its surface. The section-knife, grasped firmly but

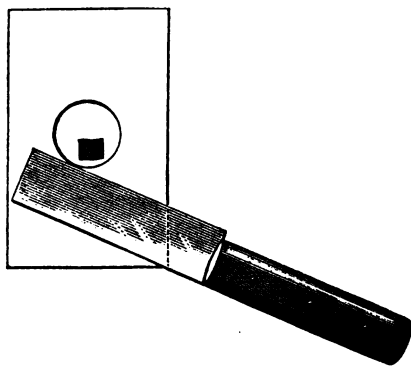


DIAGRAM SHOWING DIAGONAL POSITION OF KNIFE IN COMMENCING TO MAKE A SECTION.

lightly in the right hand, is to be laid flat upon the cutting plate of the machine, so as to occupy the diagonal position shown in the figure. Two fingers of the left hand are now laid gently upon the back of the blade, so as to

give it an equable support, whilst the knife with a rapid motion is pushed in the combined direction of *forwards* and to the *left*, so that the blade in cutting the tissue will pass through it from point to heel. Thus it will be observed that the stroke of the knife is *from* the operator—a far easier and more effective mode of cutting than the reverse plan. The blade of the knife, having the section just cut, either floating in a small pool of spirit on its surface or adhering thereto, must now be immersed in the basin of water, when by a little very gentle agitation of the knife the section will be floated off. And now we shall find the great practical value of immersing the tissue in gum before imbedding, for no sooner is the section disengaged from the knife than the thin film of gum which separates the paraffine from it becomes dissolved, and the section will be observed gradually to subside to the bottom, leaving the paraffine floating upon the surface. After carefully wiping the knife from all shreds of paraffine, the microtome screw must again be partially revolved, more spirit applied to the tissue, and another section being cut, it must be transferred to the water as before, and so on, until a sufficient number of sections have been obtained. As to how thin the sections should be cut, no general directions can be given; each



case must be regulated by its own conditions. The denser the tissue, the thinner should the section be; whilst certain substances of loose and spongy texture do not require the sections to be particularly thin—it may be said, however, in a general way that sections, and especially animal ones, *cannot be cut too thin* so long as they remain perfect and entire. If Professor Rutherford's microtome (as made by Gardner) be employed, the head of the screw will be found to be graduated into divisions of slightly unequal value; the sections will therefore be marked by corresponding variations of thickness, so that amongst a number cut, there must be many of the exact thickness to meet the requirements of any individual case.

13. *Staining Agents*.—Before proceeding to mount the sections which have just been cut, it will be very advisable that they should be submitted to the action of some staining fluid, in order to render more clear and distinct their minute structure. Organic substances possess the property of being able to absorb various colouring matters from their solution, and to incorporate such colour into their own texture. This power of attraction is not, however, possessed by all substances indiscriminately, or to an equal extent. Some possess it in a high degree, whilst others appear to be nearly, if not

entirely, devoid of such power. Hence it follows, that if we immerse an organic tissue (one of our sections, for instance) of complex structure, in a suitable staining fluid, the tissue will not become stained in an even and uniform manner throughout, but the several portions of it will receive varying depths of colour in accordance with the varying attractive power of its several constituents. By this means we are enabled in stained sections to discriminate by their difference of shade, minute and delicate structures, which in the unstained condition it would be difficult and often impossible to differentiate. For the purpose of section-staining there are many agents in use, the most generally suitable being carmine, logwood (§ 19), and aniline blue (§ 27); whilst for special purposes chloride of gold (§ 28), pyrogallate of iron (§ 28), and several others are all of much value.

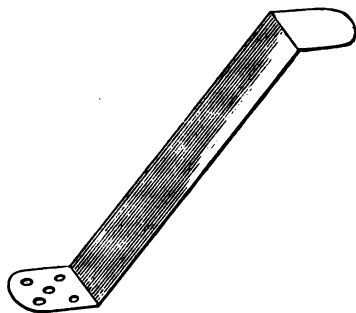
14. *Carmine Staining.*—In the case of animal sections, carmine is, as a rule, to be selected, giving as it does most satisfactory and beautiful results. Tissues may be stained with carmine by two different plans: in the first, a strong solution is used, and the tissue subjected to its action for a very short period only, whilst in the latter only very weak solutions are employed, the time of immersion being considerably prolonged. The rapid method, how-

ever, is not to be recommended, for the strong carmine acts so powerfully upon the tissue as to give the various elements comprising it no time, as it were, to exercise their power of quantitative selection, but involves the whole in one uniform degree of shadeless colour. By adopting the gradual method much better results are obtained, each portion of the tissue being now at liberty to acquire its own particular shade. Amongst the various formulæ for the preparation of carmine fluid, none can be so safely followed as that devised by Dr. Lionel Beale. It runs thus :—Place ten grains of the finest carmine in a test tube, add thirty minims of strong liquor ammonia, boil, add two ounces of distilled water, and filter ; then add two ounces of glycerine, and half an ounce of rectified spirit—this solution ought to be kept in a well-stoppered bottle. The best vessels in which to stain sections are small jars of white porcelain, capable of holding about two fluid ounces, and furnished with lids—they are much preferable to beakers or watch glasses, for owing to the white background which they afford it is very easy to watch how the staining is proceeding. The carmine solution which we have just described is both too strong and of too great density to be used in its pure *state*. It will, therefore, require to be diluted *with distilled water* before use—the most use-

ful degree of dilution being attained by adding one part of stain to seven of water. Sections may be placed in this solution for twenty-four hours, in which time they will usually be found to have acquired a sufficient depth of colour. If, however, the tissue be unusually difficult to stain, the time of immersion may be doubled, or still further prolonged, without detriment to the section.

Having prepared and filtered some of this dilute solution, say an ounce, let us proceed to stain with it those sections which we left in the basin of water (§ 12). Here we are at once met by a practical difficulty. How are the sections to be transferred from one vessel to the other? This is ordinarily effected by means of a soft camel's-hair pencil. It is a method, however, open to grave objections, for the sections so curl around the brush, and get entangled amidst its hairs, that, notwithstanding every care, valuable sections not unfrequently become torn during transit. Every difficulty at once vanishes if we substitute for the brush a small implement, which anyone can readily make for himself. All that is necessary is to take a strip of German silver, or copper, of the thickness of stout cardboard, and about seven inches in length by five-eighths of an inch in breadth. The sharp angles are to be filed off and the edges carefully smoothed, whilst at

a distance of five-eighths of an inch from each extremity the end must be turned up so as to form an angle of about  $35^{\circ}$ . One end must be left plain, whilst the other, with the aid of a punch or drill, is to be pierced with five holes about the thickness of a stocking needle\* (see Figure). If we now dip the perforated end of this spoon into the water containing the sec-



SECTION SPOON.

tions, and gently agitate it, the sections will rise from the bottom and float about. The

\* Dr. Klein describes a kind of "lifter," made by bending some German-silver wire, but as no drawing accompanies his description it is not easy to form a clear idea as to the form of this instrument. In the recent and philosophical work of Schäfer, a lifter is figured, which consists of a wire stem, having attached to its end a spade-like blade. It will be observed that the spoon described in the text differs from this lifter in having one end perforated, and in this consists the real value of the implement.

spoon is now brought under one of them, and being steadily lifted up the water flows downwards through its apertures, and the section smoothly spreading itself out upon the spoon, may be gently lifted out of the water, and on the spoon being dipped into the staining fluid the section at once floats off. By this simple means sections, however large, thin, or delicate, may with ease be conveyed from one fluid to another, with the utmost certainty of their not being injured during the process. The sections having been in the carmine fluid for about twenty-four hours, as much of the liquor as is possible must be gently poured off, and its place supplied by a freshly-filtered mixture of five drops of glacial-acetic acid to one ounce of water, when in a few moments the carmine will become permanently *fixed* in the tissue, and the process of staining be complete.

15. *Mounting Media*.—The further treatment of the stained sections will entirely depend upon the nature of the medium in which it is intended to mount them. There are a variety of fluids in use for this purpose, the principal being dilute alcohol (§ 26), dammar, or Canada balsam (§ 22-23), and glycerine. These, however, cannot be used indiscriminately, each possessing certain special properties which render it suitable for use with particular

classes of objects only. Thus, weak spirit, having no tendency to increase the transparency of objects, can advantageously be used with such only as are already perfectly transparent. It is also more suitable for the preservation of vegetable tissues (when the retention of colour is no object) than animal, since with the latter it has a tendency after a while to cause a kind of granular disintegration, which ultimately destroys much of the usefulness of the preparation. Dammar and Canada balsam, on the other hand, possess very great refractive power, so that they are of great service in mounting objects which require their transparency to be much increased. For this reason they are not well adapted to the preservation of very delicate or transparent tissues (unless previously stained), the minute details of which become almost entirely obliterated when mounted in them. The chief advantage possessed by these resinous media is, that tissues mounted in them undergo no alteration, even after the lapse of many years. Glycerine, in respect of its clarifying powers, occupies an intermediate position between spirit and balsam, being much more refractive than the former, infinitely less so than the latter. It is, therefore, of very great value for the preservation of such tissues as possess a medium

degree of transparency, and which would become obscured if mounted in spirit, or have their outlines rendered indistinct if preserved in balsam. It is of the utmost value for mounting unstained anatomical sections which, when put up in this medium, reveal such minute details of structure as would readily have escaped observation had any other agent been employed. It may also be used with stained sections, but in this case the sections should be of extreme thinness, otherwise the refractive power of the glycerine will be insufficient to render them thoroughly transparent. The great drawback to the use of glycerine is the extreme difficulty experienced in preventing its escape from beneath the covering glass, for it unfortunately possesses such great penetrating power that no cement hitherto devised can be thoroughly depended upon for withstanding its solvent action for any considerable length of time. Attention to the instructions presently to be given (§ 16) will, however, reduce this risk of leakage to a minimum. In the use of glycerine Dr. Carpenter's caution must ever be borne in mind, viz., that, as carbonate of lime is in time dissolved by glycerine, this agent ought never to be employed for the preservation of objects containing such salt.

16. *Mounting in Glycerine.*—To illustrate the method of using this medium we will



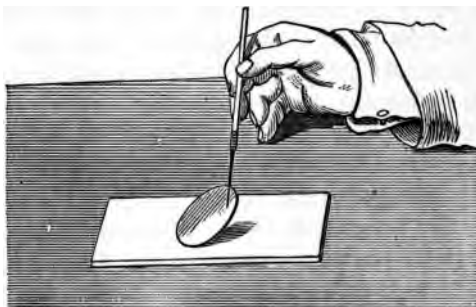
mount our present sections in glycerine. In the first place we shall require a deep watch-glass, which is to be half filled with glycerine diluted with an equal amount of distilled water. By means of the spoon, one or more sections may be transferred into this, either directly from the acetic acid solution (§ 14), or if, since cutting, they have been preserved in spirit, they should first undergo a short immersion in a large vessel full of water. The watch-glass should now be covered with an inverted wine-glass, and put away for some hours, in order that the sections may become thoroughly saturated with the dilute glycerine. When this has been accomplished, a slide must be cleaned, and one of the sections, with the aid of the *unpierced* end of the spoon, be transferred to its centre.\* As the kind of section with which

\* The appearance of a slide is vastly improved if the preparation be placed *exactly* in its centre. This may readily be done in the following manner:—Take some very finely-powdered Prussian blue, and rub it up in a mortar with a little of the weak gum solution (§ 11), so as to form a thin blue pigment. A quantity of this should be made, so as always to be at hand. A slide having been cleaned, the *best surface* is to be selected, and on the *reverse* side, by means of the self-centring turn-table, a small circle is to be drawn with a camel's-hair pencil, charged with the pigment. In the centre of this ring, but on the opposite side of the slide, the section is to be placed, when it of course will occupy a position exactly central. *When the slide comes to be finished, the blue ring may easily be removed with a wet rag.*

we are now dealing is, or ought to be, of extreme thinness, no cell (§ 26) is necessary. After tilting up one end of the slide, so as to drain off as much of the weak glycerine as possible, a drop of Price's best glycerine must, with a glass rod or pipette, be allowed to fall gently upon the section, so as to avoid the formation of air-bubbles. If any of these, however, should be produced, they must be removed with the point of a needle set in a wooden handle,\* and the slide then covered with a small bell-glass (or wine-glass). A circular cover is now to be cleaned with a soft handkerchief, and after gently blowing from it any adhering fibres of lint, etc., it will be advisable to hold the side of the glass which is to come into contact with the preparation close to the mouth, and breathe upon it, so as to cover it with moisture. The cover held between the thumb and forefinger of the left hand must now be applied by its edge near to the margin

\* A *crochet-needle* holder made of bone, and which may be bought at the smallware dealers for about sixpence, makes an admirable handle for microscopical needles. At one extremity there is a small cavity, closed with a cap, for the storage of reserve needles, whilst the other end terminates in a metal tip, provided with a crucial slit and central perforation for the reception of the needle in actual use, and so arranged that, by means of a small screw-nut, needles of various sizes may be firmly held in position.

of the preparation, and the surface of the cover directed in an inclined manner over it. Beneath the overhanging edge of the cover the point of the needle, held in the right hand, is now to be inserted (see Figure). By gently lowering the needle, the cover will come into gradual contact with the slide, driving before it a minute wave of glycerine, in which any air-bubbles that may have become developed are usually carried off.



METHOD OF APPLYING COVER.

A very considerable degree of tact, however, is required to perform this little operation, simple as it may appear, for the retreating wave of glycerine not unfrequently floats out the section, either wholly or partially, from beneath the cover. Air-bubbles, also (the *bêtes noires* of this process), are exceedingly likely to arise. When this happens the best plan to adopt is, *by means* of the needle point, gently to raise

and remove the cover, apply another drop of glycerine to the section, and cover *with a fresh piece of thin glass*. It will now be necessary to remove any superfluous glycerine which may have collected around and near the cover. The great bulk must be wiped away by means of a camel's-hair pencil, slightly wetted between the lips, any remaining stickiness being removed with a bit of blotting paper which has been slightly damped. With a very small camel's-hair pencil, charged with solution of gelatine, a ring must be made round the margin of the cover, of sufficient breadth to take in a small tract of both cover and slide. As this cement is perfectly miscible with glycerine, it readily unites with any of that fluid which may ooze from beneath the cover, and which, in the case of any of the ordinary varnishes, would act as a fatal obstacle to perfect adhesion. To make the cement, take half an ounce of Nelson's opaque gelatine, put in a small beaker, add sufficient cold water to cover it, and allow the mixture to remain until the gelatine has become thoroughly soaked. The water is now poured off, and heat applied until the gelatine becomes fluid, when three drops of creosote should be well stirred in, and the fluid mixture transferred to a small bottle to solidify. Before use, this compound must be rendered liquid by

immersing the bottle containing it in a cup of warm water. When the ring of gelatine has become quite set and dry (which will not take long), every trace of glycerine must be carefully removed from the cover and its neighbourhood, by gently swabbing these parts with a large camel's-hair pencil dipped in methylated spirit. After drying the slide a ring of Bell's microscopical cement may be applied over the gelatine, and, when this is dry, another coat is to be laid on. If it be desired to give to the slide a neat and tasteful appearance, it is a very easy matter, by means of the turntable, to lay on a final ring of Brunswick black or white zinc cement (§ 24). Every care has now been taken to render our preparation permanent; but, to make assurance doubly sure, it will be well to follow Dr. Carpenter's advice, and, every year or so, to lay on a thin coating of good gold size.\*

\* If square covers be employed, they may be fixed to the slide by a simple method much in vogue in Germany. A thin wax taper is to be lighted, and being partially inverted for a few seconds, the wax surrounding the wick will become melted. After the slide has been freed from excess of glycerine, a drop of this heated wax is allowed to fall upon each corner of the cover, and a line of the melted wax run along the margins of the cover between these points, so as perfectly to surround it. If a good coat of white zinc cement be subsequently laid over the wax a very durable, and not unornamental, line of union *will have been formed.*

17. *Uses of Freezing Microtome.*—Our preceding consideration of the method of employing the microtome in conjunction with paraffine as an imbedding agent (§ 11), will have formed a very suitable introduction to the study of the somewhat more complicated process of imbedding the tissue in gum, for section in the freezing microtome. This method is of the utmost value to the practical histologist, for by its means he is enabled with ease to possess himself of perfect sections of several structures, the cutting of which, before the introduction of this process, was always a matter of difficulty and anxiety. The freezing microtome is especially valuable for the section of such substances as from their extreme delicacy are liable to be injured by being imbedded in paraffine—for instance, the delicate villi of the intestines become very frequently, by the use of paraffine, denuded of their epithelium, and the villi themselves not seldom become torn off or otherwise damaged. The great value of the method is also very well seen in the treatment of those tissues which, like the lung, are of such loose and spongy texture as to offer insufficient resistance to the knife unless their interstices have previously been filled up with some solid yet easily cut material. As the space at our command is

strictly limited, we are precluded from entering as fully into this branch of section-cutting as the importance of the subject demands and our own inclination would lead us. To those who wish to become thoroughly conversant with the full value of this method we cannot do better than recommend the perusal of Professor Rutherford's *Practical Histology*, 2nd edition, than which, on the whole subject of physiological microscopy, no treatise with which we are acquainted is at once so plain, practical, and profound.

18. *Employment of Freezing Microtome.*—A very suitable object with which to demonstrate the method of using this form of microtome will be afforded us by a portion of intestine, say of the ileum of a cat or dog. Suppose we have some of this in methylated spirit—let us select a piece of about half an inch in length. Our first care will be to deprive this of its spirit; for so long as the tissue remains impregnated with alcohol it would, of course, be impossible to freeze it. We will, therefore, throw it into a large basinful of water, and leave it there for twenty-four hours, during which time it would be as well to change the water once or twice. We shall now require a strong solution of gum. This, which should have been made some time previously, may be

prepared by placing a quantity, say three or four ounces, of ordinary gum arabic in a glass beaker, and adding sufficient water to cover it—the mixture must be stirred occasionally with a glass rod until solution has taken place, which will be in a few days. If *necessary* a little more water may be added, but so long as the gum will pour from vessel to vessel it cannot well be made too strong. Mucilage, by keeping, is very apt to become sour and mouldy—this may be prevented by adding to each ounce of the water with which it is prepared about half a grain of salicylic acid. We now pour some of this mucilage into a small vessel—an egg cup will answer very well—and into it transfer the piece of ileum from the water. Here we must allow it to remain for a time sufficient to permit of its becoming thoroughly saturated with the gum, for which purpose some hours will be necessary. When this soaking has been accomplished we will prepare the microtome, which we will assume to be Rutherford's. In the first place it will be necessary to remove the plug—which is to be done by turning the handle connected with the screw until the plug rises so high in its tube that it may be grasped with the fingers and removed, when it is to be well smeared all over with sperm



oil and replaced. This is done to prevent any unpleasant adhesions taking place whilst the freezing is going on. We must next depress the plug, so as to convert the upper part of the tube into a kind of "well" of sufficient depth to hold our specimen. It will now be very advisable to look carefully into this *well*, and observe whether the plug fits accurately into the tube (§ 8) for if there be any interval between the two it will give rise to much subsequent annoyance, as the gum penetrating this interstice will there become firmly frozen into irregular patches, which will so interfere with the even gliding of the plug within its tube as to cause the former to ascend in such an irregular and jerky manner as to be utterly destructive of all accuracy in the cutting. If this defect be observed, it may be at once remedied by dropping a small quantity of gently heated paraffine into the *well*, which will effectually close up any fissures. The microtome, by means of its clamping arrangement, must now be firmly attached to the table, and a suitable vessel be placed on the floor beneath it, so that it may catch the water which will issue from the waste-pipe of the apparatus. The next requirement is a supply of block ice and finely powdered salt. A lump of the ice must be wrapped in a towel, and crushed into small

pieces; these, by means of a large mortar, are to be further reduced to a very *fine powder*. Any attempt to hurry over this troublesome part of the operation will lead to future disappointment, for unless the ice be used in a very fine powder great delay (at least) in the freezing will be the result. With the aid of a small spoon the ice and salt are in alternate spoonfuls to be conveyed into the freezing-box of the machine, great care being taken that the cavity under the cutting-plate and around the tube be thoroughly packed, after which the uncovered portion of the box should also be well filled. The *well* is now to be filled with the strong gum to within a little distance of its top, and a piece of sheet gutta-percha (such as shoe soles are made of) being applied over the well, and kept in position by a weight, we must wait until the freezing commences. In a short time we shall notice that the gum has acquired a thick muddy appearance. The tissue must now, by means of the forceps, be transferred to the well, and there placed in such a position that the sections, when cut, shall run in the desired direction. After more gum has, if necessary, been added, so as completely to cover the tissue, the well is again to be covered, and attention given to the freezing-box. As the mixture which this contains

becomes melted, it must constantly be renewed, care being at the same time taken that the mouth of the discharge-pipe be kept quite free, otherwise water accumulating in the box, the freezing mixture will degenerate into a useless puddle. When the gum becomes sufficiently hard to cut, this must be done much in the same manner as if paraffine had been used (§ 12). In this case, however, no fluid will be required, or must be used, to wet the knife with, and especial care must be taken that in disengaging the sections from the knife into the water they be not torn. These sections often adhere very tenaciously to the blade, but if a little patience be exercised the water will soon float them off in safety—much more safely than if any attempt be made to liberate them prematurely. There is one circumstance connected with the use of the freezing microtome which is rather annoying. The moisture of the breath and atmosphere is apt to become condensed on the cutting-plate, and here, mixed with accidental smears of gum, it becomes frozen into a jagged and irregular sheet of ice, which not only seriously interferes with the smooth play of the knife, but also constitutes a real peril to its edge. As this evil cannot be avoided, all we can do is, by constant wiping, to keep the cutting-plate clean and free from this

accumulation. This is best done with a bit of soft rag *just moistened* with spirit, but this must not come into contact with any portion of the cylinder of frozen gum, else it will instantly thaw it. When using the freezing microtome it is always advisable to wear an apron, otherwise our clothes may receive considerable damage from the constant splashing of the salt water, as it falls from the waste-pipe into the vessel beneath it. After use, the microtome must be well washed in plenty of cold water till every trace of salt be removed, for if any of this remain it will quickly corrode the brass-work of the instrument. The plug and screw, as also the section-knife, should be well smeared with Rangoon oil before the machine is put away.

19. *Logwood Staining*.—The employment of logwood as a staining agent is now becoming very general. It acts much in the same manner as carmine, but the violet colour which it produces is by many thought to be of a more soft and agreeable character than that due to the action of carmine. A valuable and very convenient property also which it possesses is that it stains tissues very rapidly, and this without interfering with that differential kind of coloration (§ 14) upon which the chief value of all staining pro-

cesses depends. A simple method of preparing the logwood fluid is to mix an aqueous solution of extract of logwood with a solution of alum (1 to 8) till the deep impure red colour has become violet, and then to filter the mixture (Frey). This will stain sections in about half an hour. This stain, though here mentioned for the ease with which it may be made is, as a rule, very inferior to a fluid prepared directly from hæmatoxylon, the alkaloid or active principle of logwood. As, however, it is difficult and troublesome to make the solution in this manner,\* it will be advisable for the student to purchase, ready prepared, such small quantity of the dye as he may require. Small bottles may be obtained for a few pence of Mr. Martindale, 10, New Cavendish Street, London, and from repeated trials of this solution we can recommend it as producing excellent results. It is a very strong fluid, and requires to be diluted before use. The degree to which the dilution must be carried cannot, however, be very accurately indicated, for all staining fluids of this nature possess the very undesirable property of becoming decomposed by age. After the fluid has been kept for some time, a portion of the

\* Should the student, however, determine to prepare *this solution* for himself, he will find a good formula for the purpose in Schäfer's "Practical Histology," p. 176.

colouring matter is thrown out of solution, and becomes deposited upon the sides and bottom of the vessel in which it is contained, hence the older the preparation, the weaker it will have become. As the time required for staining with logwood is but short, it is desirable that all the sections should begin to be submitted to its action at the same time, otherwise some will become more deeply stained than others. A good plan is to fill a small porcelain jar (§ 14) with filtered water, and into this transfer the sections. Whilst they are settling well down to the bottom, a mixture must be prepared of half a drachm of Martindale's solution (fresh) to one ounce of distilled water, and everything got in readiness for its immediate filtration. The water is now very gently to be poured off the sections, and if care be exercised this may be done in such a manner as to leave them undisturbed at the bottom, after removing almost every drop of water. The diluted logwood fluid must now be *immediately* filtered upon the sections, so that they may run no risk of becoming dry. In the present instance the staining may be allowed to proceed for about thirty minutes, and this will be found a convenient time for the immersion of the general run of animal sections. If the logwood fluid be not quite fresh, either a little more of it will have to be added to the water, or the time

of immersion must be prolonged until the desired depth of colour has been produced. It is well whilst the staining is going on gently to shake the vessel occasionally, so that the sections may not remain in a heap at the bottom, but all be as fully as possible exposed to the action of the dye. When the staining is judged to be complete, the logwood solution must be gently poured off, leaving the stained sections at the bottom of the jar, when they should be quickly covered with methylated spirit, which will *fix* the colour. We shall now be able to see if the coloration obtained be perfectly satisfactory. If not deep enough, it is very easy again to submit them to the action of the dye for a few minutes longer. If on the other hand, and as more frequently happens, the coloration should be too deep, the excess of colour may readily be removed by transferring the sections for a short time into some diluted acetic acid prepared by adding five drops of the glacial acid to an ounce of water. The action of this should be carefully watched, and when the colour has been reduced to the desired tint the sections may be retransferred to the methylated spirit.

20. *Absolute Alcohol*.—As we purpose mounting the sections which have just been stained, in *Canada* balsam, we will briefly consider the

preliminary treatment to which they must be submitted before this can be effected. The object of this is to abstract from the tissue all its water, for if any moisture be permitted to remain in the section it will, when mounted in balsam, become obscured and surrounded by a kind of opalescent halo, due to the imperfect penetration of the balsam into the only partially dehydrated tissue. The old-fashioned plan of dehydration was simple exposure to the air. The method now generally adopted is to bring about the same result by means of absolute alcohol. This fluid has such a strong affinity for water that tissues submitted to its influence are rapidly and effectually deprived of any water they may contain. Absolute alcohol in small quantity may be obtained from the druggist at about sixpence per ounce. It will be necessary for the student to provide himself with a little of this agent, say about two ounces, the method of using which will very shortly be explained. Absolute alcohol must be kept in a bottle with a very accurately-fitting stopper, in order to prevent its absorbing moisture from the air. For our purpose such a bottle, having a neck *as wide as possible*, is to be selected.

21. *Clove Oil*.—After being thoroughly dehydrated the sections may, in special instances



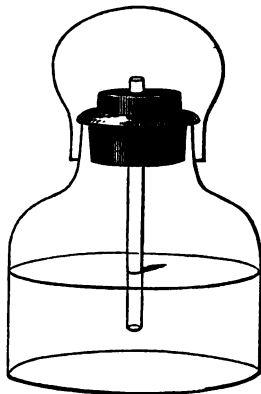
(§ 48), be at once mounted in balsam; but, as a general rule, it will be found necessary (particularly in the case of animal sections) to treat them with some clarifying agent, in order to remove the cloudiness and opacity which is (in part) due to their previous immersion in alcohol. For this purpose turpentine, or any of the essential oils, may be used: of these, oil of cloves is to be specially recommended. It is rather expensive, ranging from sixpence to one shilling per ounce; but, as a drop or two will be sufficient for preparing each slide, only a small quantity—say half an ounce or an ounce—need be procured. The most convenient vessel in which to keep the oil is one of the small test bottles used by watchmakers. These bottles are provided with a glass cap to exclude dust, and the stopper is prolonged into a glass rod, which dips into the bottle. The use of this rod and the method of employing the oil will be explained shortly.

22. *Canada Balsam*, as ordinarily met with, is a thick resinous balm of great viscosity, but readily rendered perfectly fluid by the application of heat. Formerly, sections were mounted in this medium in its pure state, but owing to the annoyance which was so constantly being experienced from the tenacity with which intruding air-bubbles were held by the viscous

medium, this plan of mounting is rapidly falling out of use.\* It is now usual to employ the balsam in a diluted condition, the two chief diluents being chloroform and benzole. As balsam, however, often contains more or less moisture, it is desirable to drive this off before adding the diluent. A very convenient way of doing so is to expose some pure balsam to the heat of a cool oven for several hours, when the

\* Although we cannot too strongly insist upon the use of chloroform-balsam wherever practicable, yet it sometimes happens in the mounting of substances of *considerable thickness*, that after all the chloroform has evaporated an insufficient amount of balsam is left behind to fill up the cavity between slide and cover. In such cases, therefore, it is advisable to use pure balsam, which may be done in the following manner. The object having been previously thoroughly dehydrated by immersion in absolute alcohol, is to be thence transferred to a little good turpentine or benzole, where it should remain until perfectly transparent. It is now to be placed in the centre of a slide which has been gently warmed, and a drop or two of *fresh* fluid balsam added, the greatest care being taken to prevent the formation of air-bubbles. Should such arise they must be touched with the point of a heated needle, which will cause them to burst and disappear. The chief difficulty of the process has yet to be encountered in the application of the cover; for it is during this procedure that the development of air-bubbles is most likely to take place. This annoyance may, however, be entirely avoided by taking the simple precaution of dipping the cover into turpentine before it is applied (§ 16), when it will be found that "you can't get air-bubbles, even if you try." The courtesy of Mr. J. A. Kay, of Chatham, enables us to give our readers the benefit of this practical "*wrinkle*."

balsam will be found to have assumed a hard, vitreous character. It should now be broken into small pieces, these put into a bottle, and some methylated chloroform added, which in a little while will completely dissolve the hardened balsam. More chloroform is then to be added, until a solution is obtained sufficiently thin to run through filtering-paper. A glass spirit lamp must now be procured, having



SPIRIT LAMP ADAPTED TO CONTAIN BALSAM.

a capacity of about two ounces, and provided with a cap. Into the wick-holder of this (which must be made of porcelain) a hollow glass tube is to be so fitted that its end dips into the lamp to within about a third of the bottom. (See Fig.) The thin chloroform balsam

is now to be filtered into this lamp, very fine filtering-paper—through which a little chloroform has first been passed—being used for the purpose. When the lamp is full it must (deprived of its cap) be put in a warm place until sufficient of the chloroform has evaporated to leave behind it a fluid of the consistence of thin syrup.

23. *Mounting in Balsam.*—Let us now return to our sections which, it will be remembered, were left in methylated spirit (§ 19). These we will now mount in balsam, and although, of course, any number may be proceeded with at the same time, yet, to avoid confusion, in the following directions one section only will be spoken of. This section then is, with the perforated spoon, to be transferred to the bottle of absolute alcohol, where it may remain for about an hour—considerably less time is *actually* required, but as from constant use the spirit becomes weakened, it is as well to be on the safe side. It must now be removed to the centre of a clean glass slip, and here the *plain* end of the spoon comes into use. If this be employed for effecting the transfer, it will be found that when the section is being removed from the alcohol it will bring along with it a small pool of the spirit. A slight touch of the needle applied to the edge of the section will

cause it to float from the spoon on to the slide, at the same time carrying the pool of alcohol with it, in which it will gently spread itself out upon the slide without the faintest risk of injury. The superfluous spirit is now to be drained off, and just as the section is becoming glazed and sodden-looking (*not dry*) we must, by means of the long glass stopper (§ 21), apply to it a large drop of clove oil. The oil, however, should not be placed *on* the section, but be allowed to drop on to the slide near to its margin. By gently tilting the slide the oil will gradually insinuate itself *beneath* the section and slowly ascend through it to the surface. The slide should now be covered with a bell-glass (or wine-glass), and about two minutes allowed for the oil thoroughly to saturate the section. As much as possible of the superfluous oil must then be drained off, and the remainder removed with blotting paper. By means of the glass rod a small quantity of chloroform balsam is now taken from the spirit lamp which contains it, and allowed gently to fall upon the section, which must then be covered with a thin glass circle in the manner previously described (§ 16). When the object is very fragile, it is a good plan, after draining off the clove oil, to apply the cover directly *upon the section*, and then to place a drop of

the balsam near to the edge of the cover. This, by capillary attraction, will speedily diffuse itself beneath the cover, flowing over and surrounding the object, without in the slightest degree disturbing its position. If, during the process of mounting, any air-bubbles arise, we may view their development with equanimity, being well assured that as the chloroform evaporates they too will quickly disappear. When the mounting is completed, the slide should be roughly labelled and placed on a warm mantel-piece for a few days to dry.

24. *Finishing the Slide.*—In the course of two or three days it will be advisable to take an old penknife, and after heating the blade in the flame of a spirit lamp, gently to run the point of it round the margin of the cover, so as to remove any excess of balsam which may have oozed from beneath it. In a few days more, any remaining balsam may be carefully scraped away with a cold knife. All remaining traces of balsam are then to be removed from around the cover by means of a rag *just moistened* with methylated spirit, or, what is better, with a mixture of equal parts of spirit and æther, after which the slide is to be thoroughly washed in cold water. The slide is now in reality finished, but, in order to give it a smart appearance, it is usual, with the assistance of the turntable, to

run a ring of coloured varnish round the covering glass. A very useful varnish for the purpose is the *white zinc cement*. To prepare this, dissolve an ounce of gum dammar in an ounce of turpentine by the aid of heat. Take one dram of oxide of zinc and an equal quantity of turpentine; rub them up together in a mortar, adding the turpentine drop by drop, so as to form a creamy mixture perfectly free from lumps or grit. One fluid ounce of the dammar solution previously made must now gradually be added, the mixture being kept constantly stirred (*Frey*). The cement, when made, should be strained through a piece of fine muslin, previously wetted with turpentine, into a small wide-necked bottle, which, instead of having a cork or stopper, should be covered with a loose metal cap. Instead of a bottle, the varnish may be kept in one of the collapsible tubes used by artists; but though this plan is highly recommended by many, it is not without its disadvantages. If the varnish becomes thick by keeping, a few drops of turpentine or benzole well stirred in will soon reduce it to a suitable consistence for use.

## PART II.

25. *Special Methods*.—Having in the preceding pages entered at some length into the general subject of section-cutting, it remains for us now to consider those special methods of preparation which the peculiarities of certain objects demand. In order to keep the bulk (and consequent price) of this manualette within due bounds, we shall, without further preface, proceed to the description of these methods, in doing which every endeavour will be made to employ such brevity of expression as may be consistent with perfect clearness of meaning. As the most convenient plan, the objects here treated of will be arranged in alphabetical succession.

26. *Bone*.—Both transverse and longitudinal sections should be prepared, the former being the prettier and most interesting. After prolonged maceration in water, all fat, etc., must be removed and the bone dried, when as thin a slice as possible is to be cut off in the desired



direction, by means of a very fine saw. If the section so obtained be placed upon a piece of smooth cork it may, with the aid of a fine file and the exercise of care, be further reduced in thickness. It is then to be laid upon a hone moistened with water, and being pressed gently and *evenly* down upon it with the tip of the finger (protected, if necessary, by a bit of cork or gutta-percha), it must be rubbed upon the stone until the desired degree of thinness has been attained. Finally, in order to remove scratches and to polish the section, it should be rubbed upon a dry hone of very fine texture, or upon a strop charged with putty-powder. After careful washing in several waters the section must be allowed thoroughly to dry, when it may be mounted by the *dry method* in the following manner:—A ring of gold-size must, by means of the turntable, be drawn in the centre of a slide, and the slide put away in a warm place for several days (the longer the better), in order that the ring may become perfectly dry and hard. When this has been accomplished the section is to be placed in the centre of the ring, and a covering circle of the requisite size having been cleaned, this must have a *thin* ring of gold-size applied round its margin. The cover is now to be placed in *position* and gently pressed down, a spring clip

being employed, if necessary, to prevent it from moving. In about twenty-four hours another layer of the varnish should be applied, and the slide afterwards finished in the manner already described (§ 24). The above method is also applicable to the preparation of sections of *teeth*, and also of *fruit-stones* and other hard bodies, which are incapable of being rendered soft enough for cutting.

As the process just described, however, is both troublesome and tedious, it is much better for ordinary purposes to have recourse to the *decalcifying method*, by which means sections in every way suitable for the examination of the essential structure of bone may be obtained with ease. To carry out this plan a piece of fresh bone should be cut into small pieces and placed in a solution made by dissolving 15 grains of pure chromic acid in 7 ounces of distilled water, to which 30 minims of nitric acid s.g. 1.420 are afterwards to be added. Here they should remain for three or four weeks, or until the bone has become sufficiently soft to cut easily, the fluid being repeatedly changed during the process. From this solution they must be transferred to methylated spirit for a few days, when a piece may be selected, imbedded in paraffine, and cut in the microtome (§ 12). Some of the sections should be

mounted, unstained, in spirit. For this purpose a cell of gold-size, as above described, must first be prepared and filled *full* of a mixture of spirit of wine one part, and distilled water three parts. Into this the section must be carefully placed and the cover applied, the same precautions for the exclusion of air-bubbles being taken which were recommended when speaking of mounting in glycerine (§ 16). When the cover is in position a ring of gold-size must be laid on, repeated when dry, and the slide afterwards finished in the ordinary manner. It will also be advisable to stain some of the sections with carmine (§ 14), or picrocarmine (§ 42), and mount them in glycerine. *Teeth* may also be treated by the decalcifying method, but in this case it must be remembered that the enamel will dissolve away.

27. *Brain*.—The best hardening fluid is that recommended by Rutherford, and is made by dissolving 15 grains of pure chromic acid and 31 grains of crystallized bichromate of potash in 43 ounces of distilled water. Small pieces of brain, which have previously been immersed for twenty-four hours in rectified spirit, should be placed in about a pint of this solution, where they must remain for five or six weeks, the fluid being repeatedly changed during the *process*. If by this time they are not suffi-

ciently hard the induration must be completed in alcohol. Sections are easily cut in the microtome by the paraffine method (§ 12). These may advantageously be stained in a solution of aniline blue, made by dissolving  $1\frac{1}{2}$  grain of aniline blue in 10 ounces of distilled water, and adding 1 drachm of rectified spirit (*Frey*). As this stain acts very rapidly two or three minutes' immersion will generally be found long enough. The sections must then be mounted in balsam (§ 23).

28. *Cartilage*.—The method to be employed in the preparation of cartilage will entirely depend upon the nature of the staining agent, to the action of which the sections are to be submitted. Thus, if the elegant *gold method* is to be followed, it is necessary that the cartilage should be perfectly fresh; whilst if any of the other staining agents are to be employed the tissue may have been previously preserved in alcohol. An excellent object on which to demonstrate the gold process is to be found in the articular cartilage of bone. It is a very easy matter to obtain from the butchers the foot of a sheep which has just been killed. The joint is to be opened, and the bones dissociated, when they will be seen to have their extremities coated with a white glistening membrane—this is the *articular cartilage*.

Exceedingly thin slices must be at once cut from it, and as only small sections are required, a sharp razor may be used for the purpose, the blade being either dry or simply wetted with distilled water. The sections as cut are to be transferred to a small quantity of a half per cent. solution of chloride of gold in a watch glass. Chloride of gold may be purchased in small glass tubes hermetically sealed, each tube containing 15 grains, and costing about 2s. If, however, the student requires only a small quantity of the staining fluid he need not be even at this small expense, for as photographers for the requirements of their art always keep on hand a standard solution of chloride of gold of the strength of one per cent., a little of this may readily be obtained, and diluted to the required degree. After the sections have been exposed to the action of the staining fluid for about ten minutes they may be transferred to a small beaker of distilled water, and exposed to diffused light for about twenty-four hours, when they must be mounted in glycerine (§ 16).

Sections of cartilage may also be examined, without being stained, in which case the field of the microscope should be only very feebly illuminated. Or carmine staining (§ 14) may be resorted to—these sections show well in *glycerine*, or if the staining be made very deep,

even Canada balsam may be employed, and with fair results.

Microscopists are indebted to Dr. Frances Elizabeth Hoggan for the description of a new method of staining, which we have found especially suited to the treatment of cartilage. The agent employed is *iron*, and the process, which is very simple, is as follows. Two fluids are necessary—(1) tincture of steel; (2) a two per cent. solution of pyrogalllic acid in alcohol. A little of the former is to be poured into a watch glass, and into this the sections, after having been previously steeped in alcohol for a few minutes, are to be placed. In about two minutes the iron solution is to be poured away and replaced by solution No. 2. In the course of a minute or two the desired depth of colour will have been produced, when the sections are to be removed, washed in distilled water, and mounted in glycerine. The results obtained by this process are very beautiful, the colour produced being a very fine neutral tint, of delightful softness. The process also answers admirably in the case of morbid tissues, and we have now in our possession some sections of ulcerated cartilage tinged by the iron method, in which the minute changes resulting from the ulcerative disintegration are brought out with wonderful distinctness.

*As the structure of cartilage differs according*

to its purpose and situation, the student will find his time profitably employed in a careful examination of the following forms ( $\alpha$ ) *hyaline*—articular and costal; ( $\beta$ ) *yellow fibro-cartilage*—epiglottis, or external ear; ( $\gamma$ ) *cellular*—ear of mouse. Sections of the *intervertebral ligaments* should also be made, in which the different kinds of cartilage may be examined side by side with each other.

29. *Coffee Berry* affords sections of great beauty. The *unroasted* berry should be soaked for hours or days in cold water until sufficiently soft; then imbedded in paraffine, and cut in the microtome (§ 12), the section being made in the direction of the long axis of the berry. Put up in glycerine, or stain rather strongly with carmine, and mount in balsam. The same method of treatment may also be applied to other hard berries or *seeds*.

30. *Fat*.—Adipose tissue may be hardened in alcohol, cut in paraffine, and mounted in glycerine. If the tissue has been injected the sections may be mounted in balsam, and are then very beautiful objects, showing the capillary network encircling the fat cells.

31. *Hair*.—Longitudinal sections are readily made by splitting the hair with a sharp razor. It is more difficult to cut the hair transversely. *This*, however, may easily be done in the fol-

lowing manner. The hairs having previously been well soaked in æther to remove all fatty matters, a sufficient number of them must be selected to form a bundle about the thickness of a crow quill. This bundle, after being tied at each extremity with a bit of thread, is to be immersed for several hours in strong gum (§ 18), to which a few drops of glycerine have been added. On removal, the bundle must be suspended by means of a thread attached to one end of it, in a warm place until sufficiently hard, when it is to be imbedded and cut in paraffine (§ 12). Each section, as cut, is to be floated off the knife into methylated spirit. From this it is with the aid of the spoon (§ 14) to be transferred to a slide, the spirit tilted off, a drop of absolute alcohol added, when, after a minute or two, this also is to be drained off, the section treated with clove oil, and the mounting completed as described in § 23.

32. *Horn* varies very much in consistence, in some instances having a cartilaginous character, whilst in others it is almost bony. In the latter case, sections will have to be ground down in the manner explained when speaking of bone (§ 26). Where the texture is less dense, recourse may be had to prolonged steeping in hot or boiling water; in some cases it will be necessary to continue the immersion



for several hours. When sufficiently soft the piece of horn may, by means of bits of soft wood, be firmly wedged into the tube of the microtome, and sections cut with a razor, or what is better, with a broad and very sharp chisel. The sections are to be put between glass slips, held together by American clips (or pegs), and put away for two or three days in order to become thoroughly dry. After well soaking in good turpentine or benzole, they must be transferred to slides, the superfluous turpentine drained off, and chloroform-balsam added, etc. (§ 23). Sections of horn should, of course, be cut in different directions, but for examination with the polariscope those cut transversely yield by far the most magnificent results. *Hoofs, whalebone*, and allied structures should also be treated by the above method.

33. *Intestine*.—The method to be pursued with *sections* has already been described (§ 18). The ileum, however, is a very pretty object when a portion of it is so mounted as to show the *villi erect*. To do this it is necessary to cement to the slide, by marine glue, a glass cell of sufficient depth. This should have been prepared some time beforehand, so that the cement may be perfectly dry and hard. The cell is now to be filled with turpentine, and the piece of ileum (having been previously passed

through methylated spirit and absolute alcohol into turpentine) is gently placed into it, having the villi uppermost; pour some pure and rather fluid balsam on the object at one end, and gradually incline the slide, so as to allow the turpentine to flow out at the opposite side of the cell, till it is full of balsam. Then take a clean cover, and having placed upon it a small streak of balsam from one end to the other, allow it gradually to fall upon the cell, so as to avoid the formation of air-bubbles (§ 17), and finish the slide in the usual manner.\* Or, the intestine may be dried, and mounted *dry*, in a cell with a blackened bottom, for examination as an opaque object.

34. *Liver*.—Small pieces of liver may be very successfully hardened by immersion in alcohol, beginning with weak spirit and ending with absolute alcohol. Cut and mount as usual.

35. *Lung* must be prepared in chromic acid (§ 5). For the cutting of sections the freezing microtome (§ 18) is of especial value, and should, therefore, be used. If, however, the student be not provided with this instrument, he must proceed as follows. A small piece of lung, previously deprived of all spirit, is to be immersed until thoroughly saturated in solu-

\* Ralf.

tion of gum (§ 18). A small mould of bibulous paper (§ 2), only just large enough to receive the piece of tissue, having been prepared and filled with the mucilage, the specimen is to be transferred to it. The mould, with its contents, is now to be placed in a saucer, into which a mixture of about 6 parts of methylated spirit and 1 part of water (*Schäfer*) is to be poured until the fluid reaches to within about a third of the top of the paper mould. In the course of several hours the surface of the mucilage will begin to whiten and solidify. As soon as this occurs more dilute spirit must be poured into the saucer, until the mould is completely submerged. In a day or two the gum will be found to have acquired a suitable consistence for cutting, when it must be removed from the spirit, the paper mould peeled off, and the mass imbedded and cut in paraffine, the sections being afterwards treated as if they had been obtained by the freezing method (§ 18). If the solidification of the gum should proceed too slowly, a few drops of pure spirit may be added to the contents of the saucer. If, on the other hand, the gum should become overhard, it will be necessary to put into the saucer a few drops of water, and repeat this until the required consistence be obtained.

36. *Muscle*.—Harden in chromic acid, and

cut in paraffine. Transverse sections may be made to show the shape of the fibrils. Longitudinal sections will only be required in the case of injected tissues, when such sections will be found very elegant, showing, as they do, the elongated meshes of capillaries running between and around the muscular fasciculi. Mount in glycerine or balsam. To see the transverse striæ characteristic of voluntary muscle, a very good plan is to take a bit of pork (cooked or fresh), and by means of needles to tease it out into the finest possible shreds. If these be examined in water or glycerine, the markings will be shown very perfectly.

37. *Orange-peel*, common object though it be, is not to be despised by the microscopist. Transverse sections must be prepared by the gum method (§ 35). These sections are not to be subjected to the action of alcohol (as this would destroy the colour), but after *drying* between glass slides they must be soaked in turpentine and mounted in balsam. We shall then have a good view of the large globular glands whose office it is to secrete that essential oil upon which the odour of the orange depends.

38. *Ovary* may be prepared in the same manner as liver (§ 34). Sections, which are to be cut in paraffine, may be stained with

carmine, and mounted in glycerine or balsam. Apart from all scientific value, we know of no slide for the microscope which, even as a mere object of show, surpasses in beauty a well-prepared section of *injected* ovary, showing the wondrous Graafian vesicles, surrounded by their meandering capillaries.

39. *Porcupine Quill*.—Soften in hot water, cut in paraffine, and mount in balsam. Much (in our opinion *too* much) lauded as an object for the polariscope.

40. *Potato*.—From the large amount of water which it contains thin sections cannot be cut from the potato in its natural state. It must, therefore, be partially desiccated, either by immersion in methylated spirit for a few days or by exposure to the air. Sections may then readily be obtained by imbedding and cutting in paraffine. Such sections mounted in balsam are very beautiful, the starch being seen *in situ*, whilst if polarized light be employed each granule gives out its characteristic black cross.

41. *Rush* is to be prepared and cut as orange-peel (§ 37). Transverse sections of this "weed" furnish slides of the most exquisite beauty.

42. *Skin*.—To prepare skin for section a piece is to be selected which, after having been *boiled* for a few seconds in vinegar, must be

stretched out on a bit of flat wood, and being maintained in position by pins be allowed to remain until thoroughly dry. Then imbed in paraffine, and cut *exceedingly* thin transverse sections. These may be stained in carmine, but more beautiful results are obtained if micro-carmine be employed. Sections of skin, when stained by this agent, are much increased both in beauty and instructiveness; for the several constituents of the tissue becoming tinged with different colours are readily distinguishable from each other, whilst the contrast of colouring forms a pleasing picture to the eye. The method of preparing micro-carmine is very simple, though it sometimes yields a solution not altogether satisfactory. The best formula with which we are acquainted is that given by Rutherford,\* and if due care be taken in following it, out failure will generally be avoided. "Take 100 c.c. of a saturated solution of picric acid. Prepare an ammoniacal solution of carmine, by dissolving 1 gramme in a few c.c. water, with the aid of excess of ammonia and heat. Boil the picric acid solution on a sand bath, and when boiling add the carmine solution. Evaporate the mixture to dryness. Dissolve the residue in 100 c.c. water, and filter. A clear solution

\* "Practical Histology," 2nd edit. p. 173.

ought to be obtained ; if not, add some more ammonia, evaporate, and dissolve as before." Sections may be exposed to the action of this fluid for a period varying from fifteen to thirty minutes, then rapidly washed in water, and mounted in glycerine. They may also be mounted in balsam, care being taken in that case to shorten as much as possible the period of their immersion in alcohol, so that no risk may be run of the picric acid stain being dissolved out.

If it is intended to study the structure of the skin with anything like thoroughness, portions must of course be examined from different localities, in order that its several varieties and peculiarities may be observed. Thus the *sudoriferous*, or sweat glands, may be found in the sole of the foot, whilst the *sebaceous* glands are to be sought in the skin of the nose. The *papillæ* are well represented at the tips of the fingers,\* whilst the structure of the shaft of the *hair*, together with that of the follicle within which its root is enclosed, as also the

\* It is well, in connection with these papillæ, to bear in mind a fact pointed out by *Frey*, namely, that the tips of the fingers frequently become, *post-mortem*, the seat of extensive natural injections ; hence, in sections from this region, we frequently obtain good views of distended capillaries without having been at the trouble of previously injecting them.—*Frey*, "Microscopical Technology."

muscles by which it is moved, are to be studied in sections of skin from the scalp or other suitable locality.

43. *Spinal Cord*.—The spinal cord, say of a cat or a dog (or if procurable, of man), after being cut into pieces about half an inch in length, may be hardened in the usual chromic acid fluid (§ 5). As it is peculiarly liable to overharden and become uselessly brittle, the process must be carefully watched. Its further treatment is the same as that of brain. These sections may be stained very satisfactorily by the *ink process*, for communicating details of which we are indebted to the kindness of Dr. Paul, of Liverpool. The agent usually employed is Stephenson's blue-black ink, which, for this purpose, must be quite fresh. As in the case of carmine, two methods of staining may be adopted—either rapid, by using concentrated solutions, or more prolonged, according to degree of dilution. For the reasons previously given (§ 14), slow methods of staining are always to be preferred, as yielding the most beautiful results, yet, for the purposes of preliminary investigation, it is often convenient to have recourse to the quick process. To carry out the latter plan, an ink solution of the strength 1 in 5—10 parts of water is to be freshly prepared, and the sections exposed



to its action for a few minutes. For gradual staining the dilution must be carried to 1 in 30—50, and the time of immersion prolonged to several hours, the sections being occasionally examined during the staining, so that they may be removed just as they have acquired the desired tint. When a satisfactory coloration has been obtained, the preparations should be mounted in dammar or balsam (§ 23). One advantage of this method of staining is, that definition is almost as good by artificial light as by day.

44. *Sponge* may readily be cut after being tightly compressed between two bits of cork; or its interstices may be filled up by immersion either in melted paraffine (§ 11) or in strong gum (§ 18), and then cut as usual.

45. *Stomach* requires no special method of hardening (chromic acid). Sections should always, when practicable, be cut in the freezing microtome. In default of this, proceed in the manner as directed for lung (§ 35). Both vertical and horizontal sections will, of course, be required. If the preparation has been injected, the latter are particularly beautiful. Stain with carmine or aniline blue (§ 27), and mount—if for very close study, in glycerine—if injected and for a “show” slide, use balsam.

46. *Tongue*.—Harden in chromic acid, imbed and cut transverse sections in paraffine. As, however, the paraffine is apt to get entangled amongst the *papillæ*, whence it is afterwards with difficulty dislodged, it will be as well before imbedding to soak the tongue in strong gum for a *few minutes*, and afterwards immerse in alcohol till the gum becomes hardened, so that the delicate *papillæ* may thus be protected from the paraffine by a surface-coating of gum. The best staining agent is picro-carminé (§ 42). Sections of *cat's* tongue near the root, when thus stained, furnish splendid objects. Sections should also be made of the *taste-bulbs*, found on the tongues of rabbits. These are small oval prominences, situated one on each side of the upper surface of the tongue near its root. They should be snipped off with scissors, and vertical sections made in the direction of their long axis. Stain with carmine or picro-carminé, and mount in glycerine or balsam.

47. *Vegetable Ivory*.—After prolonged soaking in cold water may readily be cut in the microtome. The sections should be mounted in balsam, and though not usually regarded as polariscopic objects, nevertheless, when examined with the *selenite*, yield very good colours.

48. *Wood*.—Shavings of extreme thinness

may be cut from large pieces or blocks of timber, by means of a very sharp plane. In this way very good sections may be procured of most of the common woods, as oak, mahogany, "glandular wood" of pine, etc. Where, however, the material to be operated upon takes the form of stems, roots, etc., of no great thickness, they should, after having been reduced to a suitable consistence (§ 4), be imbedded in paraffine, and cut in the microtome. Before imbedding it must not be forgotten to immerse the wood to be cut in weak gum-water (§ 11), this precaution being of great importance, especially in the case of stems, etc., the bark of which is at all rough and sinuous. If the sections are to be mounted *unstained*, they are usually put up in weak spirit (§ 26). A very general method also of dealing with this class of objects is to mount them *dry* (§ 26). This plan, however, cannot be recommended, for however thin the sections may be, the outlines, when this process is adopted, always present a disagreeable black or blurred appearance. To avoid this we may have recourse to Canada balsam, but the ordinary method of employing it must be slightly modified, a drop of chloroform being substituted for the clove oil (§ 23), otherwise this latter agent will cause the *section* to become so transparent as to render

minute details of structure difficult to recognize. A better plan, perhaps, is to stain the section with carmine or logwood, and mount in balsam by the ordinary process. The best course to follow, however, especially in the case of transverse sections, is the *double staining* method.\* For this purpose the sections in the first place must be subjected to the action of a solution of chloride of lime ( $\frac{1}{4}$  oz. to a pint of water) until they become thoroughly bleached. They must then be soaked in a solution of hyposulphite of soda (one drachm to four ounces of water) for an hour, and after being washed for some hours, in several changes of water, are to be transferred for a short time to methylated spirit. Some red staining-fluid is now to be prepared by dissolving half a grain of Magenta crystals in one ounce of methylated spirit. A little of this solution being poured into a small vessel of white porcelain (§ 14), the sections are to be immersed in the dye for about thirty minutes. They are now to be removed, and after *rapid* rinsing in methylated spirit to remove all superfluous colour, they must be placed in a blue staining fluid made by dissolving half a

\* See a paper by Mr. Stiles in the "Pharmaceutical Journal;" also, "Monthly Microscopical Journal" for August, 1875.

grain of aniline blue in one drachm of distilled water, adding ten minims of dilute nitric acid, and afterwards sufficient methylated spirit to make two fluid ounces. The sections must be permitted to remain in this solution for a very short time only, one to three minutes being generally sufficient, for as the action of the dye is very energetic, it will, if too long exposure be allowed, completely obliterate the previous coloration by the magenta. After being again *rapidly* rinsed in methylated spirit, as much of this as possible must be drained off, and the sections put into oil of *cajeput*, whence, in an hour, they may be transferred to spirits of turpentine, and, after a short soaking, mounted in balsam.

If the student will carefully carry out the above process, his trouble will be amply repaid by the beautiful results obtained, for by its means he may, with ease, prepare for himself a series of slides of such value as to constitute a worthy addition to his cabinet.

The preceding list by no means represents all the objects, sections of which will be found interesting to the microscopic student. Such was not its purpose—had it been so, the enumeration might have been prolonged almost indefinitely. The end in view was to *bring* under the notice of the reader only those

substances the cutting of which is accompanied by difficulty; and even of this class the space at our disposal has been so limited that we have been unwillingly compelled to pass over many, and dwell only on such as possess a typical character.















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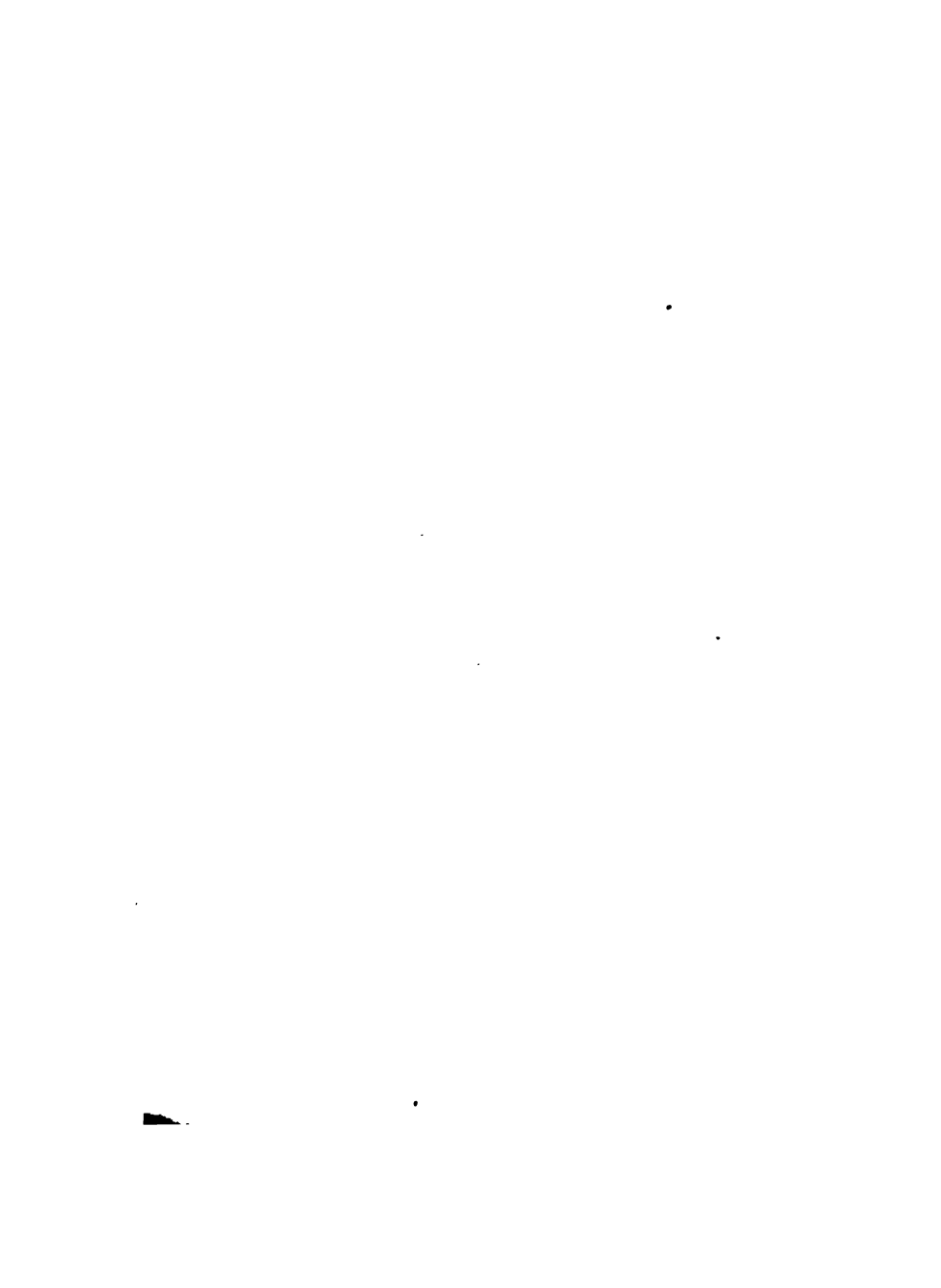




















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